Eur päisches Patentamt | European Patent Office | Office eur péen des brevets

EP00/03913

4

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

99120211.0

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE, 19/06/00
LA HAYE, LE

Eur päisches Patentamt
Eur pean Patent Office
Office européen des brevets

Blatt 2 der Besch inigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.: 99120211.0
Demande n°:

Anmeldetag:
Date of filing: 09/10/99
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
Evotec BioSystems AG
22525 Hamburg
GERMANY

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Method of diagnosing or treating Alzheimer's disease on basis of increased cerebrospinal fluid levels of nerve growth factor

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
/

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du depôt:

Bemerkungen:
Remarks:
Remarques:

Methods of diagnosing or treating Alzheimer's disease on basis of increased cerebrospinal fluid levels of nerve growth factor

Alzheimer's disease (AD), first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neuropsychiatric disorder which begins with short term memory loss and proceeds to loss of cognitive functions, disorientation, impairment of judgement and reasoning and, ultimately, dementia. It is the most common form of dementia. The neuropathology is characterized by the formation in brain of amyloid plaques and neurofibrillary tangles. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. Moreover, as adults, born during the population boom of the 1940's and 1950's, approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Familial forms of AD are genetically heterogeneous, but most with early onset are linked to mutations in the presenilin genes *PSEN1* and *PSEN2*, as well as to mutations of the amyloid precursor gene *APP*. The majority of AD patients have no obvious family history and are classified as sporadic AD. For this late onset AD, several putative genetic risk factors have been reported. Among these the ApoE-epsilon 4 (ApoE 4) has been widely confirmed to confer increased risk for AD. Inheritance of ApoE4 and other risk factors are neither necessary nor sufficient to cause AD. In contrast to the APP- and PSEN mutations which increase the production of Aβ, the principal component of senile plaques in AD brain, the ApoE variant most likely influences Aβ accumulation by modulating clearance and degradation of the peptide.

In the search for biochemical changes in patients with neuropsychiatric and neurodegenerative disorders analysis of cerebrospinal fluid (CSF) may be a useful method, since the CSF is continuous with the extracellular fluid of the brain. Therefore, a plurality of studies aiming at the analysis of the central nervous system (CNS) specific proteins in CSF were performed in order to find biochemical markers for neuronal and synaptic function and pathology in degenerative brain disorders.

Nerve growth factor (NGF) is one of the neurotrophic agents that promote differentiation or support the survival and functioning of some populations of neurons, influencing their

effects not only on the peripheral sensory and sympathetic neurons but also on the central neurons. The pathophysiological role of NGF in the human nervous system, especially in relation to neuropsychiatric disorders, has not been fully understood yet. It is known that patients with acute multiple sclerosis (MS), traumatic brain injury or hypertensive cerebral hemorrhage show higher NGF levels in the CSF and NGF has trophic roles in regenerating axons in the CNS.

To determine the pathophysiological roles of NGF in the human CNS with special reference to neuropsychiatric disorders, levels of NGF in CSF from patients with the following neurodegenerative disorders have been examined by Nisho et al. (Clinica Chimica Acta 275, 93 – 98, 1998) using a highly sensitive two-site enzyme immunoassay:

(i) Parkinson's disease
(ii) Progressive supranuclear palsy
(iii) Sporadic olivo-ponto-cerebellar atrophy
(iv) Spinocerebellar ataxia 3 / Machado-Joseph disease
(v) Dentato-rubro-pallido-luysian atrophy

However, Nisho et al. did not examine any patients suffering from Alzheimer's disease.

As AD is a growing social and medical problem, there is a strong need for methods of diagnosing or prognosing said disease in subjects as well as for methods of treatment.

In one aspect, the invention features a method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:
determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
and comparing said level to a reference value representing a known disease or health status,
thereby diagnosing or prognosing said Alzheimer's disease in said subject, or determining whether said subject is at increased risk of developing Alzheimer's disease.

It is preferred to use a body fluid, preferably cerebrospinal fluid, as said sample. An increase of a level of nerve growth factor in cerebrospinal fluid from a subject relative to

said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

In a further aspect, the invention features a method of monitoring progression of Alzheimer's disease in a subject, comprising:
determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
and comparing said level to a reference value representing a known disease or health status, thereby monitoring progression of said Alzheimer's disease in said subject.

It is particularly preferred to further compare a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in said sample with a level of at least one of said substances in a series of samples taken from said subject over a period of time.

In still a further aspect, the invention features a method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
and comparing said level to a reference value representing a known disease or health status,
thereby evaluating said treatment for said Alzheimer's disease.

In a further preferred embodiment, additionally a level of neurotrophin 3 (NT-3) and/or of a transcription product of a gene coding for neurotrophin 3 is determined with the goal of diagnosing, prognosing, evaluating the risk of developing, evaluating a treatment of, or monitoring the progression of Alzheimer's disease.

In another aspect, the invention features a kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor or a transcription product of a gene coding for nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein an increased level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor compared to a reference value representing a known health status;
or a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor similar or equal to a reference value representing a known disease status
indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

Additionally, said kit preferably further comprises at least one reagent which selectively detects neurotrophin 3 (NT-3) or a transcription product of a gene coding for neurotrophin 3. Combined testing of NGF and NT-3 is a valuable tool in the diagnosis, prognosis, or risk evaluation of Alzheimer's disease (see example 3 and table 1).

In another aspect, the invention features a method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

It might be further preferred to administer to said subject in a therapeutically effective amount an agent or agents which directly or indirectly reduce an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, its non-coding regulatory elements, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In still another aspect, the invention features the use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

In still another aspect, the invention features a composition for use as a medicament comprising (i) a first agent which directly or indirectly reduces an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly reduces an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, its non-coding regulatory elements, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In a further aspect, the invention features the use of a composition for the manufacture of a medicament for treating Alzheimer's disease, said composition comprising (i) a first agent which directly or indirectly reduces an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly reduces an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, its non-coding regulatory elements, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

The invention further features a method for identifying an agent that directly or indirectly affects an activity, or a level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or a level, or both said activity and level, of at least one of said substances before and after said contacting.

Figure 1 relates to example 1 and depicts that CSF levels of NGF are significantly elevated in the AD group, as compared to both the group consisting of patients with major depression (DE) as well as to the control group (CTR). Levels (pg/ml) are given in mean ± SEM. Asterisk (*, **) indicate significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p < 0.001$; ** AD versus CTR, $p < 0.001$. NGF concentrations in CSF of the AD group amounted to 8.79 +/- 0.72 pg/ml (mean +/- SEM, range: 3.29 to 14.95, n = 23), compared to 4.07 +/- 0.50 pg/ml in the DE group (range: 2.42 to 9.54, n = 14), and 3.49 +/- 0.51 pg/ml in the CTR group (range: 0.00 to 4.64, n = 8), respectively. The alterations in patients suffering from AD may reflect disturbances in the trophic support of specific neuronal populations, such as the basal forebrain cholinergic system. There was no apparent correlation of CSF levels of NGF with ApoE genotype (or phenotype, respectively), age, duration of AD, MMS, NOSGER or MADRS scores.

Figure 2 relates to example 2 and depicts nerve growth factor levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p = 0.002$; ** AD versus CTR, $p = 0.000$, *** DE versus CTR, $p = 0.000$. CSF levels of NGF were significantly elevated in the AD group, as compared to both the DE and the CTR group. CSF levels of NGF were also significantly elevated in the DE group, as compared to the CTR group. NGF concentrations in CSF of the AD group amounted to 8.19 +/- 0.91 pg/ml (mean +/- SEM, range: 0.00 to 23.00, n = 40), compared to 4.26 +/- 0.97 pg/ml in the DE group (range: 0.00 to 23.00, n = 22), and 1.18 +/- 0.35 pg/ml in the CTR group (range: 0.00 to 7.20, n = 32), respectively.

Figure 3 relates to example 3 and depicts neurotrophin 3 (NT-3) levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects (CTR). CSF levels of NT-3 were determined to define a cut-off value to be used in the combined tests shown in table 1. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * DE versus AD, $p = 0.005$; ** DE versus CTR, $p = 0.000$; *** AD versus CTR, $p = 0.010$. CSF levels of NT-3 were significantly elevated in the DE group, as compared to both the AD and the CTR group. CSF levels of NT-3 were slightly, but significantly, elevated in the AD group, as compared to the CTR group. NT-3 concentrations in CSF of the DE group were 25.8 +/- 4.3 pg/ml (mean +/- SEM, range: 0.0 to 87.0, n =23), compared to 14.0 +/- 1.6 pg/ml in the AD group (range: 0.0 to 41.0, n = 39), and 10.5 +/- 1.6 pg/ml in the CTR group (range: 0.0 to 67.0, n = 63), respectively.

Table 1 relates to examples 2 and 3. This table shows the diagnostic accuracy of spinal fluid measurements of NGF and NT-3 in Alzheimer's Disease and Major Depression in the Elderly. In NGF measurements, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using a cut-off value of ≥ 4.0 pg/ml NGF (total: n = 94, AD: n = 40, DE: n = 22, CTR: n = 32). The combined NGF/NT-3 test showed a considerable specificity for the diagnosis of AD (90.1 %), using cut-off values of ≥ 4 pg/ml NGF, and < 15 pg/ml NT-3, respectively (total: n = 57, AD: n = 24, DE: n = 18, CTR: n = 15). Testing either NGF levels or NGF and NT-3 levels with suitable cut-off criteria constitutes candidate tools for specific biochemical diagnosis of AD. Using the opposite cut-off criteria, the combination test significantly separated AD patients from elderly DE patients with a specificity of 89.7 %. Therefore, another potential use of this test is the biochemical differentiation between these two frequent disorders in the elderly.

EXAMPLE 1

In order to achieve a differential diagnosis, the study included not only patients with AD, but also such with major depression (DE). Diagnosis of probable AD was made according to criteria of the National Institute of Neuropsychiatric and Communicative Disorders and Stroke-Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA; McKhann et al., Neurology 34, 939 – 944, 1984). Patients with major depression were diagnosed according to the ICD10 (F32.0x/1x, F33.0x/1x) and DSM-IIIR (296.20-22, 296.30-32) criteria. All patients were referred to the research ward from general practitioners, neurologists and psychiatrists for diagnostic purposes and screening for clinical trials. None of the patients was institutionalized. The group of healthy control subjects (CTR) consisted of patients that underwent lumbar puncture for orthopedic or neurologic diagnostic purposes and were shown to have normal CSF cell counts, total protein levels, and absence of signs of blood barrier dysfunction or cerebral IgG synthesis, as well as absence of any cerebral disorders.

AD, DE and CTR patients were carefully examined and received a thorough clinical work-up. Psychometric testing including the Mini Mental State (MMS; Folstein et al., J. Psychiatry Res. 12, 189 – 198, 1975), as a global screening instrument for dementia, and the Nurses' Observation Scale for Geriatric Patients (NOSGER; Spiegel et al., J. Am. Geriatr. Soc. 39(4), 339 – 347, 1991) as a functional measure of dementia severity. The patients with DE showed no cognitive disturbances in the clinical examinations and the Mini Mental State scores were within the normal range. Severity of depression was rated by using the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery et al., Br. J. Psychiatry, 134: 382 – 389, 1979). Apolipoprotein (ApoE) genotyping, or, if DNA was not available, ApoE phenotyping was included in the laboratory screening in the AD patients.

CSF was obtained for diagnostic purposes in the AD and DE patients in which no lumbar puncture had been previously done during the routine diagnostic work-up. Different CSF volumes were available for the analysis of the neurotrophin proteins. This fact explains the different sample sizes for the individual measurements. All available CSF samples were used for the analyses.

The AD group was as follows: n = 23, 12 men, 11 women, mean age 63.9 +/- 13.2 SD years, range 39 – 86 years, MMS score: mean 18.6 +/- 5.6 SD.

The DE group was as follows: n = 14, 5 men, 9 women, mean age 68.2 +/- 13.6 SD years, range 47 – 86 years, MMS score: mean 28.1 +/- 0.9 SD.

The CTR group was as follows: n = 8, 5 men, 3 women, mean age 60.1 +/- 18.1 SD years, range 31 – 81 years.

AD and CTR patients were free of psychotropic medication. Patients with major depression were treated with various antidepressant drugs including serotonin reuptake inhibitors, reversible monoaminooxidase A inhibitors and tricyclics. Informed consent was taken from each patient and their caregivers before the investigation. The study was approved by the local ethics committee. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Within one week of dementia testing, CSF was obtained by lumbar puncture. To control for possible influences of a ventriculo-lumbar gradient, lumbar punctures were done between 7.30 and 8 a. m. before breakfast while patients were still lying flat. CSF samples were frozen on dry ice immediately upon withdrawal at the bedside in 0.5 ml aliquots and stored at – 85 °C until biochemical analysis.

CSF levels of NGF were measured by an ELISA as described recently (Weskamp et al., J. Neurochem. 48, 1779 – 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim) diluted in carbonate buffer pH 9.2 over night at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D-galactopyranoside was added and plates were incubated at 4 °C over night. The reaction was stopped after 1 h at RT and the flurorescent product was measured in the microtiter wells using a fluorometer (Labsystems Fluoroskan Ascent FL) (excitation wavelength: 355 nm; emission wavelength: 460 nm). The detection limit was 1.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 %.

ApoE genotyping was performed using INNO-LiPA ApoE, Innogenetics, Belgium. ApoE phenotyping was performed according to McDowell et al. (Clin. Chem. 35(10), 2070 - 2073, 1989). The use of the ApoE phenotype synonymous with the ApoE genotype in the statistical analyses seemed to be appropriate, since ApoE genotyping compared with protein phenotyping showed conflicting results in less than 2 % (Hansen et al., Clin. Chim. Acta, 224(2), 131 - 137, 1994).

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Correlation analyses were performed by multiple regression using CSF levels of neurotrophins as well as ApoE genotype (or phenotype, respectively), age, duration of the disease in AD, MMS, NOSGER and MADRS scores. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

EXAMPLE 2

The study described in example 1 has been extended to a wider panel of patients as described below in this example 2.

Diagnosis, clinical examination and treatment of patients as well as lumbar puncture were performed as described in example 1.

For NGF measurements, 94 spinal fluid samples were examined. The AD group (n = 40) consisted of 18 men and 22 women, mean age 68.8 +/- 12.4 SD years, range 39 - 88 yr, MMS score: mean 19.3 +/- 4.6 SD. The DE group (n = 22) consisted of 8 men and 12 women, mean age 69.8 +/- 12.6 SD years, range 47 - 86 yr, MMS score: mean 27.5 +/- 2.1 SD. CTR group: n = 32, 18 men, 14 women, mean age 64.0 +/- 14.9 SD years, range 29 - 96 yr.

CSF levels of NGF were measured by an ELISA as described by Weskamp et al. (J. Neurochem. 48: 1779 - 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim)

diluted in carbonate buffer pH 9.2 overnight at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D-galactopyranoside was added and plates were incubated at 4 °C overnight. The reaction was stopped after 1h at RT, and the fluorescent product was measured in the microtiter wells by using a fluorometer (Labsystems Fluoroskan Ascent FL) at 355 nm excitation and 460 nm emission wavelength. The detection limit was 0.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 % and the assay was linear over a range of 0.5 to 500 pg/ml.

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

To estimate the diagnostic accuracy of the test, a) sensitivities and b) specificities, defined as follows, were calculated: a) true positives / (true positives and false negatives), and b) true negatives / (true negatives and false positives). To estimate the probability of disease, predictive values of the tests were calculated. The positive predictive value (PPV) was defined as true positives / (true positives + false positives). The negative predictive value (NPV) was defined as true negatives / (true negatives + false negatives).

EXAMPLE 3

The purpose of this study was to check whether combined measurements of the CSF levels of NGF and neurotrophin-3 (NT-3) – which also belongs to the group of neurotrophins – improves the diagnostic accuracy of the NGF test described in example 2.

In a first step, NT-3 levels in CSF were determined to define a cut-off value to be used in the combined tests. Diagnosis, clinical examination and treatment of patients, lumbar puncture and statistical analyses were performed as described in example 2. For NT-3 measurements, 125 spinal fluid samples were examined. AD group: n = 39, 20 men, 19

women, mean age 67.2 +/- 11.5 SD years, range 39 – 86 yr, MMS score: mean 19.1 +/- 5.3 SD. DE group: n = 23, 8 men, 15 women, mean age 70.5 +/- 11.9 SD years, range 47 – 86 yr, MMS score: mean 27.2 +/- 2.5 SD. CTR group: n = 63, 35 men, 28 women, mean age 56.0 +/- 15.0 SD years, range 28 – 84 yr. NT-3 was determined by using commercially available ELISA systems (Promega, Madison, WI) according to the manufacturer's protocol. 120 µl of undiluted CSF in carbonate buffer (pH 9.7) were added to 96 well immunoplates (Nunc) at 4 °C overnight. Anti-Human-NT-3 polyclonal antibodies (pAb) were used as capture Ab. Anti-NT-3 mAb were used as reporter Ab. After incubation with a species-specific Ab (anti-rat IgG) conjugated to horseradish peroxidase (HRP) as a tertiary reactant, and washing, the solution was incubated with the chromogenic substrate TMB (3, 5, 3', 5'-tetramethylbenzidine). Absorbance was measured at 450 nm by using a microplate reader (Dynatech MR 700). NT-3 ELISA: linear range 4.7 – 300 pg/ml; cross-reaction with other neurotrophins at 10 ng/ml < 3%; detection limit 6.0 pg/ml.

57 CSF samples were available for combined NGF/NT-3 measurements. AD group: n = 24, 13 men, 11 women, mean age 64.9 +/- 12.5 SD years, range 47 – 82 yr, MMS score: mean 18.6 +/- 5.4 SD. DE group: n = 18, 7 men, 11 women, mean age 69.5 +/- 12.7 SD years, range 47 – 84 yr, MMS score: mean 27.7 +/- 2.1 SD. CTR group: n = 15, 10 men, 5 women, mean age 59.0 +/- 15.9 SD years, range 29 – 80 yr.

CLAIMS

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:
   determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
   and comparing said level to a reference value representing a known disease or health status,
   thereby diagnosing or prognosing said Alzheimer's disease in said subject, or determining whether said subject is at increased risk of developing Alzheimer's disease.

2. The method according to claim 1, wherein said sample is a body fluid, preferably cerebrospinal fluid.

3. The method according to claim 2, wherein an increase of said level of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

4. A method of monitoring progression of Alzheimer's disease in a subject, comprising:
   determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
   and comparing said level to a reference value representing a known disease or health status, thereby monitoring progression of said Alzheimer's disease in said subject.

5. A method according to claim 4, further comprising comparing a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in said sample with a level of at least one of said substances in a series of samples taken from said subject over a period of time.

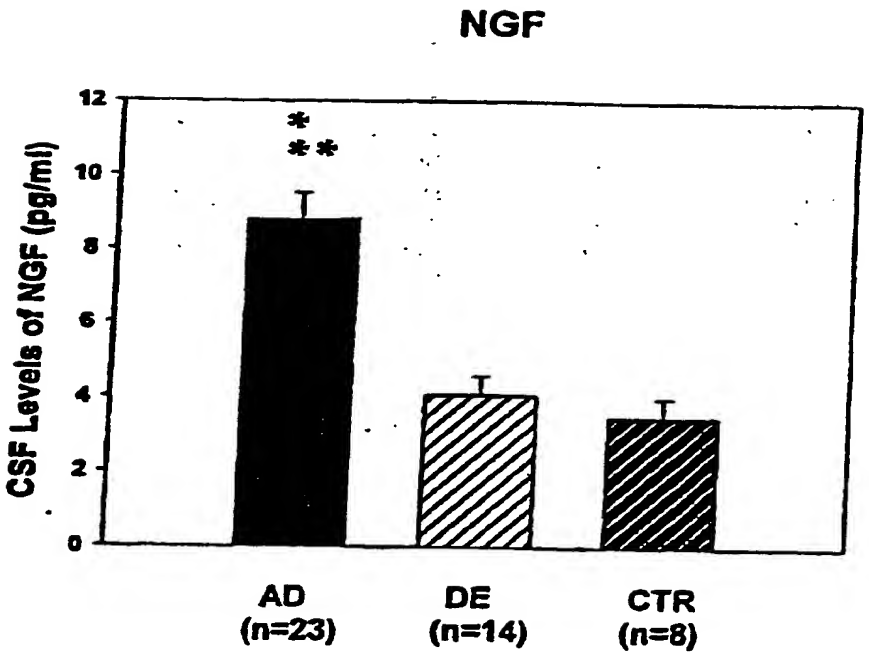
6. A method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;
and comparing said level to a reference value representing a known disease or health status,
thereby evaluating said treatment for said Alzheimer's disease.

7. A kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:
(a) at least one reagent which selectively detects nerve growth factor or a transcription product of a gene coding for nerve growth factor; and
(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by
(i) detecting a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject; and
(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,
wherein an increased level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor compared to a reference value representing a known health status;
or a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor similar or equal to a reference value representing a known disease status
indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

8. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

9. Use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

10. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:
    (a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, its non-coding regulatory elements, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;
    (b) contacting said sample with at least one agent;
    (c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.



g. 1

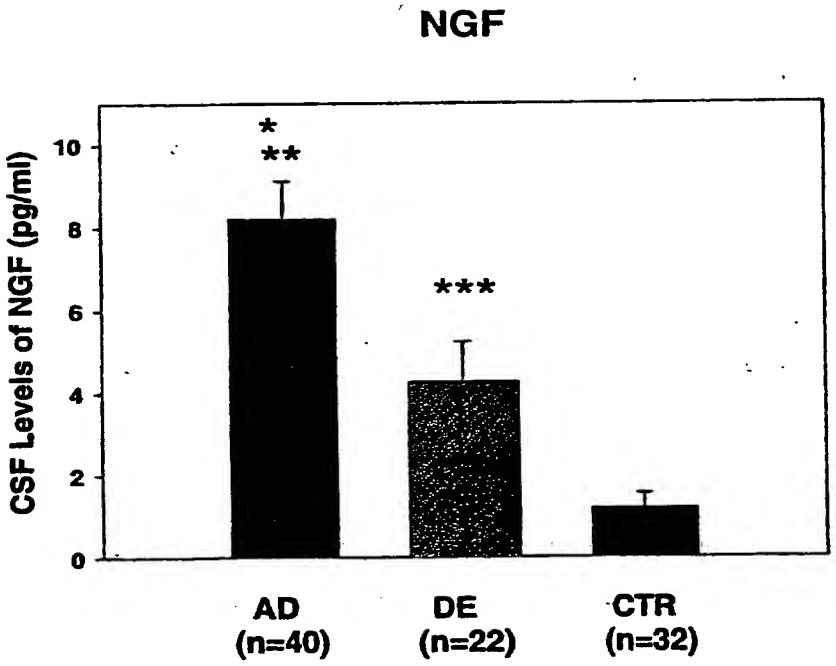


Fig. 2

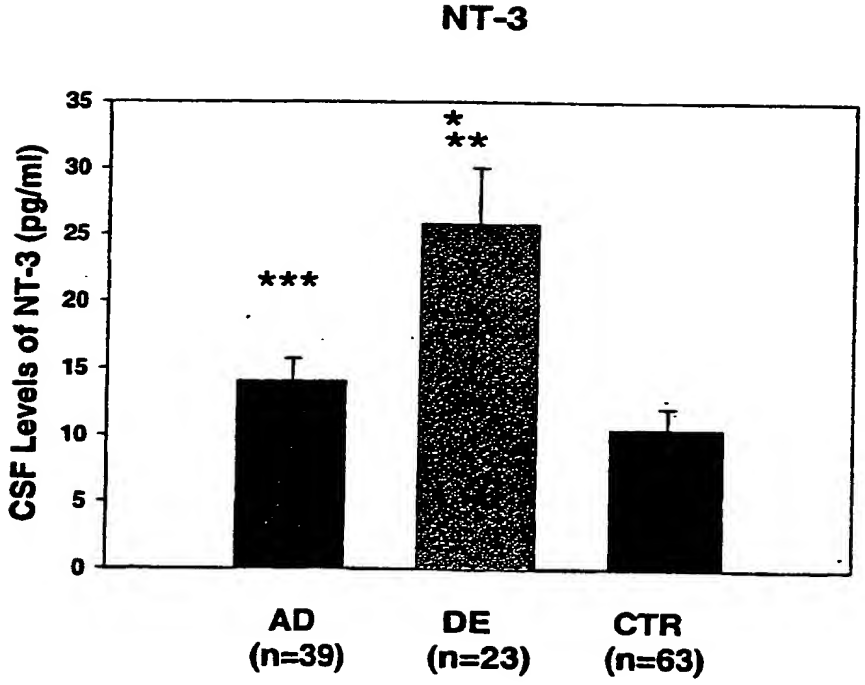

NT-3
CSF Levels of NT-3 (pg/ml)
35
30
25
20
15
10
5
0
***
*
**
AD
(n=39)
DE
(n=23)
CTR
(n=63)

Fig. 3

Spinal Fluid Measurements of NGF and NT-3: Diagnostic Accuracy in Alzheimer's Disease (AD) and Major Depression in the Eld rly (DE)

	NGF test (NGF>4 pg/ml)	NT-3 test (NT-3>15 pg/ml)	Combined NGF/NT-3 test (NGF>4 pg/ml, NT-3<15 pg/ml)	Combined NGF/NT-3 test (NT-3>15 pg/ml, NGF<4 pg/ml)
	Diagnosis of AD	**Diagnosis of DE**	**Diagnosis of AD**	**Diagnosis of DE**
Sensitivity	71,9%	73,9%	62.5%	55.5%
Specificity	79,2%	86,1%	90.1%	89.7%
Positive Predictive Value (PPV)	67,6%	50,0%	83.3%	71.4%
Negative Predictive Value (NPV)	82,4%	91,7%	76.9%	81.4%

Table 1

ABSTRACT

The invention relates to a method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level of nerve growth factor and/or of a transcription product of a gene coding for nerve growth factor in a sample from said subject;

and comparing said level to a reference value representing a known disease or health status,

thereby diagnosing or prognosing said Alzheimer's disease in said subject, or determining whether said subject is at increased risk of developing Alzheimer's disease.

Figure 1